OROTIC ACID AND ITS ANALOGUES

PART II. ON THE ALKALINE REARRANGEMENT OF 5-CARBOXYMETHYLIDENEHYDANTOIN[1, 2]

R. DEGHENGHI AND G. DANEAULT

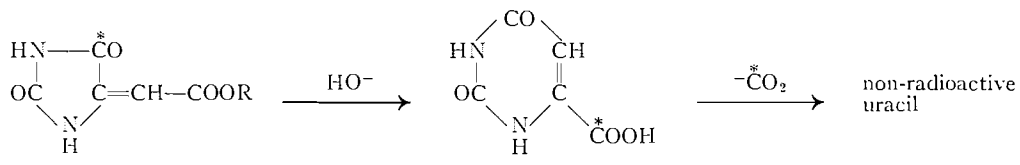
ABSTRACT

The in vitro conversion of 5-carboxymethylidenehydantoin to orotic acid is not likely to occur in vivo since the two substances have antagonistic effects (2). A sulphur analogue of the above hydantoin has been prepared and shown not to undergo alkaline rearrangement to the corresponding metathiazine structure. The mechanism of orotic acid formation is discussed.

Since its discovery in 1905 by Biscaro and Belloni (3), orotic acid (II) has aroused an enormous interest among biochemists (see in particular (4) and (5)) for its importance as a pyrimidine precursor of nucleic acids. A large amount of synthetic work has been done on orotic acid itself (6) and on its analogues (7), but comparatively little attention has been given to the mechanisms of its formation, from a purely chemical point of view.

At first orotic acid was believed to be a seven-membered ring compound (3), but it was soon shown to be uracil-6-carboxylic acid (8, 9). Its synthesis from ethyl oxaloacetate and urea was erroneously believed to give directly ethyl orotate (10); later the ester was proved to be in fact 5-carbethoxymethylidenehydantoin (11). A similar error was made by Biltz and Kramer (12), who studied the alkaline decomposition of a diazoacetate derivative of alloxan to give again the hydantoin and not the six-membered ring ester.

All these facts prompted us to reinvestigate the mechanism of the formation of orotic acid and we chose at first the reaction of ethyl oxaloacetate and urea (III → I), as described by Müller (13) and discussed by Nyc and Mitchell (14). The latter authors postulated a mechanism which involves opening of the hydantoin ring between positions three and four to give the hydantoic acid which closes again to give the pyrimidine. The reaction was followed spectrophotometrically in alkaline solution and it was found that orotic acid is formed previous to acidification. The hydantoic acid was not isolated, though in other unsaturated hydantoins evidence of the open-chain compound has been obtained (14). That an open-chain-ring tautomerism exists in the hydantoin conversion to orotic acid was clearly demonstrated by Langley (15), who was able to show that a C^{14} carbon atom in position four in the hydantoin ring is present as a carboxyl in the orotic acid; this was confirmed by the quantitative recovery of the radioactivity in the CO_2 following decarboxylation (the remaining uracil being inactive). We were interested



(I) (II)

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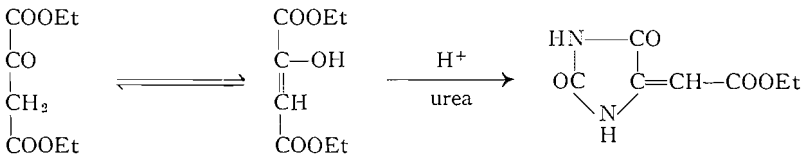
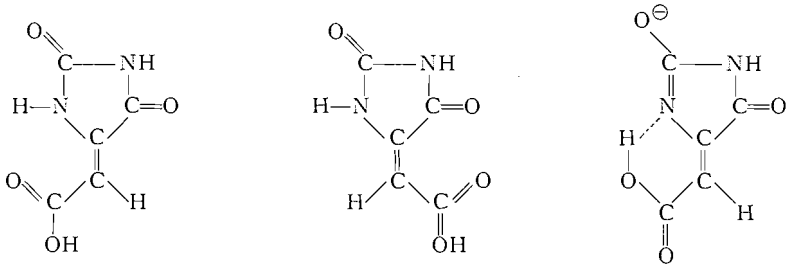
[2]*Part I: See reference 1.*

to investigate (*a*) why the hydantoin is the first intermediate when urea condenses with oxaloacetic ester, (*b*) whether the same kind of ring enlargements were possible with other five-membered ring substances, and (*c*) the isolation of possible intermediates of the above rearrangement (I → II).



(III) (IV) (I)

Oxaloacetic acid and its esters (III) are present in the pure state or in non-polar solvents (16, 17) as the enol form (IV) which may exist in the *trans* (hydroxy-fumaric) or *cis* (hydroxy-maleic) form. It is formally the latter which, after condensation with urea, gives the five-membered ring hydantoin. In fact, one could reasonably expect ethyl orotate only from the *trans* (fumaric) form of the intermediate hydantoic ester (ureidofumaric acid). For the same reason one could expect a cis–trans isomerization to occur previous to or during the alkaline conversion of the hydantoin to orotic acid. We were able to isolate two isomeric forms of the hydantoin (V, VI), one of which has not been described previously, by eluting the potassium salt of I through a column of cation-exchange resin (cf. Experimental). Proof of the structure was obtained by ultraviolet spectra, which showed two peaks at 235 mμ and 294 mμ characteristic of hydantoin structure (cf. 14), by infrared spectra, by the facile conversion of one isomer to the other, and by the fact that both substances gave, upon reduction with sodium amalgam, the same hydantoin-5-acetic acid. Furthermore, we were led to attribute tentatively structure V to the lower melting isomer, on the basis that the increase of the infrared frequency of the carbonyl in position four and the decrease of the C=O frequency in position two, together with the shift of the OH frequency due to hydrogen bonding, are better explained by structures of the type VII. Therefore the complete mechanism

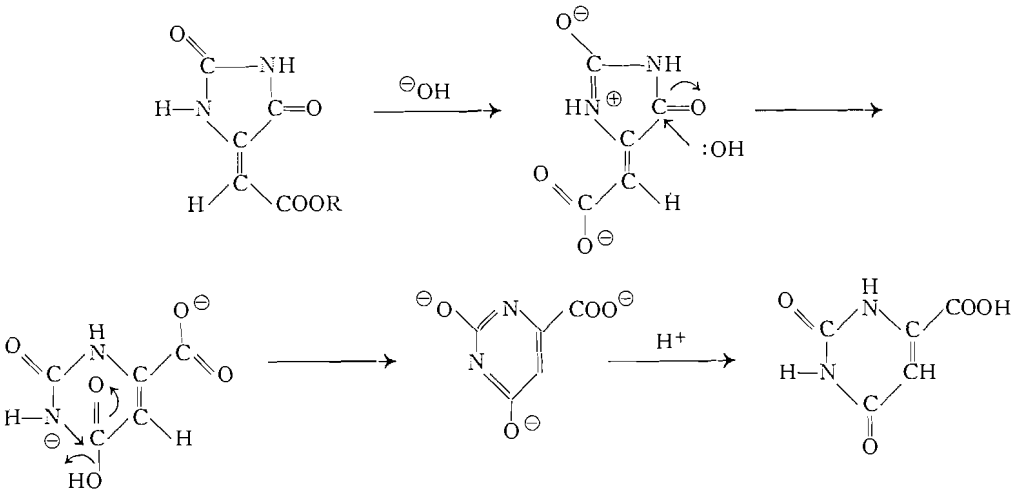


(V) (VI) (VII)

for the alkaline conversion of I to orotic acid may reasonably assume the following form going through the steps: (*a*) cis–trans isomerization,[3] (*b*) alkaline cleavage, and (*c*) aromatization to the more stable pyrimidine structure.[4]

[3]*The reversible addition of the elements of water to the double bond (cf. 19) is a possible mechanism for this isomerization.*

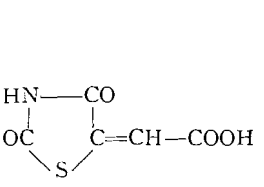
[4]*Orotic acid is essentially a hydroxy pyrimidine in alkaline medium (cf. 18).*



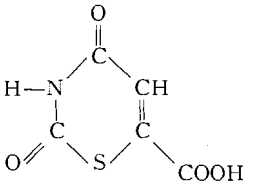
For a parallel experiment with a parent compound, we chose the corresponding pseudo-thio-hydantoin (VIII) which we synthesized in good yields from thiourea and maleic anhydride through the saturated thiazolidinedione-5-acetic acid. The ultraviolet spectrum of the new substance VIII in ethanol resembles closely that of 5-carboxymethylidene-hydantoin (cyclic, five-membered structure) and the infrared spectrum of the corresponding methyl or ethyl esters shows the four expected bands at 1772 cm^{-1} (4-CO), 1735 cm^{-1} (2-CO), 1690 cm^{-1}, and 1620 cm^{-1} (α,β-unsaturated ester). The free acid had a similar spectrum but with two more bands (KBr) at 1785 and 1700 cm^{-1} which could possibly arise from dimerization[5] (20). The free acid has a remarkable stability; it was recovered unchanged after being refluxed with an aqueous solution of $HClO_4$ and, when subjected to the same conditions for the orotic acid alkaline rearrangement, the starting material or its monopotassium salt were recovered again in high yield (cf. Experimental).

The potassium salt[6] by elution through a cationic exchange resin gave, like the above hydantoin, an isomer whose cyclic structure was evidenced by ultraviolet absorption, and which could be converted to the other form by crystallization from boiling water. Both isomers were reduced to the same saturated acid by the action of sodium amalgam.

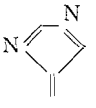
The fact that in the case of the five-membered ring sulphur compound a ring enlargement to the corresponding metathiazine structure (IX) was not achieved, in spite of



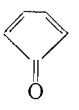
(VIII)



(IX)



(X)



(XI)

[5]*For a discussion about carbonyl band splitting, cf. (26).*
[6]*Cf. (9) for the remarkable stability of potassium orotate.*

the isomerization, may be explained by considering the stability of structures of the type X which could arise from the enolic form of the hydantoin (21). The polarity of the exocyclic double bond (conjugated with the acid) would be strongly opposed in the structure X as it is in the case of 1,2-cyclopentenediones (enol form XI) (22) which, accordingly, exist only in the keto form. With the sulphur compound evidently no enol structure of the type XI (in alkaline solution) would be possible, thus explaining the observed stability of the five-membered thiazolidine ring.

It may be mentioned here that the in vitro conversion of 5-carboxymethylidene-hydantoin to orotic acid is not likely to occur in vivo, since the two substances show antagonistic effects following subcutaneous administration in the rat (2). An account of the biological activity of the sulphur analogue of the above hydantoin will be reported elsewhere.

EXPERIMENTAL[7]

2-Imino-4-oxo-5-thiazolidineacetic Acid

This compound was prepared in 72% yield from thiourea, according to the method of Andreasch (23) reported by Flett and Gardner (24), m.p. 252–253° dec. (reported to decompose above 220°). ν_{max}^{KBr} 3300, 3150 cm^{-1} (OH, NH); 2400 cm^{-1} (broad); 1690, 1645 cm^{-1} (C=N and C=O). Calc. for $C_5H_6N_2O_3S$: C, 34.48%; H, 3.47%; N, 16.09%; S, 18.40%. Found: C, 34.62%; H, 3.71%; N, 16.34%; S, 18.28%.

2,4-Dioxothiazolidine-5-acetic Acid

A solution of 63.237 g of 2-imino-4-oxo-5-thiazolidineacetic acid was refluxed for 2 hours with 630 ml of 20% sulphuric acid. Upon cooling, 42.6 g of 2,4-dioxothiazolidine-5-acetic acid crystallized out, m.p. 168° (lit. 168–169° (25)).

Extraction of the mother liquors with ethyl acetate and evaporation of the solvent *in vacuo* gave another 19.9 g of less pure product, m.p. 163–168°. Total yield 98%. ν_{max}^{KBr} 3200, 3080 cm^{-1} (OH, NH); 1760, 1740 cm^{-1} (4 and 2 C=O); 1700 cm^{-1} (acid C=O).

5-Carboxymethylidene-2,4-dioxothiazolidine (VIII)

A mixture of 26.25 g of 2,4-dioxothiazolidine-5-acetic acid in 90 ml of acetic acid containing 8.2 ml of bromine was refluxed for 2 hours. Hydrogen bromide was evolved and from the clear solution, upon cooling, the product crystallized out in 60% yield. The crystals were collected on a filter, washed with a little acetic acid and water, m.p. 248° dec. A sample was recrystallized from water three times for analysis; needles, m.p. 252–253° dec. Calc. for $C_5H_3NO_4S$: C, 34.68%; H, 1.75%; N, 8.09%; S, 18.51%. Found: C, 34.80%; H, 1.92%; N, 7.98%; S, 18.30%. λ_{max}^{EtOH} 236 mμ (ε 4360), 302 mμ (ε 6320). ν_{max}^{KBr} 3240, 3080, 2950 (OH, NH); 1775, 1758 cm^{-1} and 1725, 1700 cm^{-1} (split carbonyls); 1670 and 1622 cm^{-1} (α,β-unsaturated acid).

The *methyl ester* was prepared by refluxing the acid (0.5 g) in 10 ml of methanol and 0.5 ml of sulphuric acid for 1 hour. Dilution with water gave 5-carbomethoxymethylidene-2,4-dioxothiazolidine, m.p. 160–162°, in 80% yield. The product was recrystallized from water for analysis, m.p. 163–164°. Calc. for $C_6H_5O_4NS$: C, 38.49%; H, 2.69%; N, 7.48%; S, 17.13%. Found: C, 38.24%; H, 2.89%; N, 7.66%; S, 17.21%. ν_{max}^{KBr} 3250 cm^{-1} (NH); 1770 and 1732 cm^{-1} (4 and 2 C=O); 1690 and 1618 cm^{-1} (α,β-unsaturated CO).

The *ethyl ester* was prepared in an analogous manner, m.p. 128–130°, λ_{max}^{EtOH} 240 mμ

[7]*Melting points, taken in evacuated capillaries, are corrected. Analyses by Schwarzkopf Microanalytical Laboratories, Woodside, N.Y. The infrared spectra were taken with a Beckman IR-4 spectrophotometer* (NaCl *optics).*

(ϵ 3940), 305 mμ (ϵ 6000). ν_{max}^{KBr} 3300 (NH); 1770 and 1720 cm^{-1} (4- and 2- CO); 1690 and 1630 cm^{-1} (α,β-unsaturated CO).

Stability of 5-Carboxymethylidene-2,4-dioxothiazolidine in Acidic Conditions

To a solution of VIII, 0.449 g in 20 ml of water, was added 2 ml of 72% perchloric acid and the whole was refluxed for 30 minutes. Upon cooling, 0.3 g of the starting material (VIII), m.p. 248° dec., crystallized out. The product was identified by infrared spectra and mixed melting point with authentic material.

Stability of 5-Carboxymethylidene-2,4-dioxothiazolidine in Alkaline Conditions

A solution of VIII, 0.693 g in 15 ml of 1 *N* KOH, was kept 30 minutes at 100°. After cooling, 8 ml of 1 *N* HCl was added. The monopotassium salt of VIII crystallized out; needles, m.p. 270° dec. (with previous browning). Calc. for $C_5H_2NO_4SK$: C, 28.43%; H, 0.95%; N, 6.63%. Found: C, 28.55%; H, 1.02%; N, 6.69%. λ_{max}^{EtOH} 235 mμ (ϵ 1330), 298 mμ (ϵ 2600). ν_{max}^{KBr} 2550 (broad); 1730, 1690, 1630, 1580 cm^{-1} (4- and 2-CO, α,β-unsaturated ionized acid). The same salt (infrared and mixed melting point comparison) was obtained when the unsaturated acid (VIII) was neutralized with 1 *N* KOH in the cold, followed by concentration of the solution *in vacuo*.

Lower Melting 5-Carboxymethylidene-2,4-dioxothiazolidine

A solution of 0.782 of the potassium salt of VIII in water was passed through a column of cation exchange resin Amberlite I.R.-120 (H). The aqueous fractions were extracted with ethyl acetate and the organic solvent was evaporated *in vacuo* to give a crystalline residue (0.7 g), m.p. 200–205° dec. ν_{max}^{KBr} 3400, 3220, 3080 (OH, NH); 1768 and 1722 (4 and 2 C=O); 1690 (shoulder); 1670 and 1615 cm^{-1} (α,β-unsaturated CO). λ_{max}^{EtOH} 240 mμ (ϵ 3840), 303 mμ (ϵ 4000). When this substance was crystallized from water, the original higher melting isomer was obtained, and identified by infrared and mixed melting-point determinations.

Reduction of 5-Carboxymethylidene-2,4-dioxothiazolidine

To a suspension of 0.759 g of VIII in 25 ml of water, stirred in an ice-water bath in a CO_2 atmosphere, 25 g of 2% sodium amalgam was added piecemeal.

After 2 hours the clear solution was decanted from the mercury, acidified with 20% sulphuric acid, and extracted with ethyl acetate. Evaporation of the solvent gave 217 mg of 2,4-dioxothiazolidine-5-acetic acid, identified by mixed melting point and infrared determination with authentic material.

When the *lower melting isomer* was reduced in a similar way, the same product, 2,4-dioxothiazolidine-5-acetic acid, was obtained in similar yield.

5-Carbethoxymethylidenehydantoin (I, R = Et)

This substance was prepared according to the method of Müller (13) from ethyl-oxaloacetate and urea. The yields were in the 10–20% range, m.p. 184–186° (reported 188°). λ_{max}^{EtOH} 235 mμ (ϵ 7920), 300 mμ (ϵ 7500), ν_{max}^{KBr} 3250 (NH); 1800, 1760 (4- and 2-CO); 1695 (C=N), 1680, 1640 cm^{-1} (shoulder) (α,β-unsaturated CO).

5-Carboxymethylidenehydantoin (I, R = H)

This acid was prepared by careful hydrolysis of the corresponding ester (I, R = Et), 184 mg, dissolved in 2.3 ml of ethanol, by addition of 0.1 g KOH in 0.4 ml water. The yellow solid formed was dissolved in 5.6 ml of water. Acidification with concentrated HCl precipitated the product, m.p. above 300° with gradual decomposition. λ_{max}^{EtOH} 237 mμ

(ϵ 7800), 300 mμ (13000). ν_{max}^{KBr} 3550, 3300, 3100 cm^{-1} (OH, NH); 1790 and 1745 cm^{-1} (4 and 2 C=O); 1680 (broad); 1638 cm^{-1} (shoulder) (α,β-unsaturated C=O). A lower melting isomer was obtained by dissolving the above acid in 1 *N* KOH and by eluting the slightly alkaline solution through a column of Amberlite I.R.-120 (H) (cation exchange resin).

The aqueous fractions combined were extracted with ethyl acetate, the solvent removed *in vacuo* to give an acid, m.p. 250° dec. ν_{max}^{KBr} 3250 (broad); 2995 (bonded OH, NH); 1800 cm^{-1} (4-CO in β,γ-unsaturated ring), 1725 (2 CO or C=N), 1690 cm^{-1} (acid CO). λ_{max}^{EtOH} 235 mμ (ϵ 5150), 294 mμ (ϵ 8850). When this substance was recrystallized from water, the higher melting isomer was obtained (infrared spectra identical). Both isomers gave, upon reduction with Na/Hg (compare the above described procedure), the same 5-hydantoin acetic acid, m.p. 212° dec. (lit. 214° (14)).

Orotic Acid

This substance was obtained by the alkaline rearrangement of 5-carboxymethylidenehydantoin according to Nyc and Mitchell (14), m.p. 320° dec. ν_{max}^{KBr} 3580, 3150, 3050 cm^{-1} (OH, NH); 1740 (shoulder); 1715 and 1680 cm^{-1} (broad) (C=N, C=O). The infrared spectrum was identical with that of an authentic sample.

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